

CLAIMS

WHAT IS CLAIMED IS:

1. A composition comprising an orthogonal aminoacyl-tRNA synthetase (O-RS), wherein the O-RS preferentially aminoacylates an O-tRNA with a redox active amino acid.
2. The composition of claim 1, wherein the O-RS comprises an amino acid sequence comprising SEQ ID NO.: 1, or a conservative variation thereof.
3. The composition of claim 1, wherein the O-RS preferentially aminoacylates the O-tRNA with an efficiency of at least 50% of the efficiency of a polypeptide comprising an amino acid sequence of SEQ ID NO.: 1.
4. The composition of claim 1, wherein the O-RS is derived from a *Methanococcus jannaschii*.
5. The composition of claim 1, comprising a cell.
6. The composition of claim 5, wherein the cell is an *E. coli* cell.
7. The composition of claim 1, comprising a translation system.
8. A cell comprising a translation system, wherein the translation system comprises:
an orthogonal -tRNA (O-tRNA);
an orthogonal aminoacyl-tRNA synthetase (O-RS); and,
a redox active amino acid;
wherein the O-tRNA recognizes a first selector codon, and the O-RS preferentially aminoacylates the O-tRNA with the first redox active amino acid.
9. The cell of claim 8, wherein the O-RS preferentially aminoacylates the O-tRNA with an efficiency of at least 50% of the efficiency of a polypeptide comprising an amino acid sequence of SEQ ID NO.: 1.
10. The cell of claim 8, wherein the O-tRNA comprises or is encoded by a polynucleotide sequence as set forth in SEQ ID NO.: 2, or a complementary polynucleotide sequence thereof, and wherein the O-RS comprises an amino acid sequence comprising SEQ ID NO.: 1, or a conservative variation thereof.
11. The cell of claim 8, wherein the cell further comprises an additional different O-tRNA/O-RS pair and unnatural amino acid, wherein the O-tRNA recognizes a second

Best Available Copy

selector codon and the O-RS preferentially aminoacylates the O-tRNA with the second unnatural amino acid.

12. The cell of claim 8, wherein the cell is a non-eukaryotic cell.
13. The cell of claim 12, wherein the non-eukaryotic cell is an *E. coli* cell.
14. The cell of claim 8, further comprising a nucleic acid that comprises a polynucleotide that encodes a polypeptide of interest, wherein the polynucleotide comprises a selector codon that is recognized by the O-tRNA.
15. An *E. coli* cell, comprising:
 - an orthogonal tRNA (O-tRNA);
 - an orthogonal aminoacyl- tRNA synthetase (O-RS), wherein the O-RS preferentially aminoacylates the O-tRNA with a redox active amino acid;
 - the redox active amino acid; and,
 - a nucleic acid that encodes a polypeptide of interest, wherein the nucleic acid comprises the selector codon that is recognized by the O-tRNA.
16. The *E. coli* cell of claim 15, wherein the O-RS preferentially aminoacylates the O-tRNA with an efficiency of at least 50% of the efficiency of a polypeptide comprising an amino acid sequence of SEQ ID NO.: 1.
17. An artificial polypeptide comprising SEQ ID NO. 1.
18. An artificial polynucleotide that encodes a polypeptide of claim 17.
19. A vector comprising or encoding a polynucleotide of claim 18.
20. The vector of claim 19, wherein the vector comprises a plasmid, a cosmid, a phage, or a virus.
21. The vector of claim 19, wherein the vector is an expression vector.
22. A cell comprising the vector of claim 19.
23. A method for identifying an orthogonal-aminoacyl-tRNA synthetase for use with a O-tRNA that utilizes a redox amino acid, the method comprising:
 - subjecting to selection a population of cells of a first species, wherein the cells each comprise:

- 1) a member of a plurality of aminoacyl-tRNA synthetases (RSs);

2) the orthogonal tRNA (O-tRNA) derived from one or more species;
and,
3) a polynucleotide that encodes a selection marker and comprises at least one selector codon;
wherein cells that are enhanced in suppression efficiency as compared to cells lacking or comprising a reduced amount of the member of the plurality of RSs that comprises an active RS that aminoacylates the O-tRNA; and,
selecting the active RS that aminoacylates the O-tRNA with the redox active amino acid, thereby providing the orthogonal -aminoacyl-tRNA synthetase for use with the O-tRNA.

24. The method of claim 23, wherein the selection comprises a positive selection and the selection marker comprises a positive selection marker.

25. The method of claim 23, wherein the plurality of RSs comprise mutant RSs, RSs derived from one or more species other than the first species or both mutant RSs and RSs derived from a species other than the first species.

26. An orthogonal aminoacyl-tRNA synthetase identified by the method of claim 23.

27. A method of producing a protein in a cell with a redox active amino acid at a specified position, the method comprising:
growing, in an appropriate medium, the cell, where the cell comprises a nucleic acid that comprises at least one selector codon and encodes a protein; and,
providing the redox active amino acid;
wherein the cell further comprises:
an orthogonal -tRNA (O-tRNA) that functions in the cell and recognizes the selector codon; and,
an orthogonal aminoacyl-tRNA synthetase (O-RS) that preferentially aminoacylates the O-tRNA with the redox active amino acid; and,
incorporating the redox active amino acid into the specified position in the protein during translation of the nucleic acid with the at least one selector codon, thereby producing the protein.

28. The method of claim 27, wherein the O-RS comprises a amino acid sequence which comprises SEQ ID NO.: 1.

29. The method of claim 27, wherein the cell is a non-eukaryotic cell.
30. The method of claim 29, wherein the non-eukaryotic cell is an *E. coli* cell.
31. A composition comprising a protein, wherein the protein comprises a redox active amino acid.
32. The composition of claim 31, wherein the redox active amino acid is selected from the group consisting of: a 3,4-dihydroxy-L-phenylalanine (DHP), a 3,4,5-trihydroxy-L-phenylalanine, a 3-nitro-tyrosine, a 4-nitro-phenylalanine, and a 3-thiol-tyrosine.
33. The composition of claim 31, wherein the protein comprises an amino acid sequence that is at least 75% identical to that of a wild-type therapeutic protein, a diagnostic protein, an industrial enzyme, or portion thereof.
34. The composition of claim 31, wherein the composition comprises a pharmaceutically acceptable carrier.

Best Available Copy